III. IN THE SPECIFICATION (CLEAN SHEET)

(¶ on page 2, lines 21-26)

The present invention provides a method of preparing Troponin I. This method comprises protecting sulfhydryl groups of reduced Troponin I, particularly recombinant TnI. In a preferred embodiment of the invention, the free sulfhydryl groups are protected by sulfitolyzation (*e.g.*, via reaction with sodium sulfite) of Troponin I expressed in a bacterial expression system. Protection of the sulfhydryl groups during Troponin I preparation obviates the costly need for maintaining reducing conditions throughout protein preparation, purification, and storage.

(¶ on page 3, line 16)

Figure 3. Summary of recombinant Troponin preparation.

(¶ on page 3, line 23-28)

Figure 6. SDS-PAGE analysis troponin lot after anion exchange steps no. 1 and no. 2 in 16% tris-glycine gel, under reducing conditions. A-H refer to lanes in the SDS-PAGE gel. A. Sulfitolyzed troponin Lot 3L4 standard; B. solubilized inclusion bodies; C. sulfitolyzed inclusion bodies (**AEX** No. 1 load); D. anion exchange no. 1 flowthrough; E. anion exchange no. 1 salt eulate; F. anion exchange no. 2 load; G. anion exchange no. 2 flowthrough; and H. anion exchange no. 2 100mM NaCl eluate.

(¶ on page 4, lines 11-14)

Figure 11. SDS-PAGE analysis of sulfitolyzed troponin reduction with dithiothreitol for 45 mins. at ambient temperature. One mg/ml TnI and six M urea, 25 mM tris, 0.15 M NaCl ph 7.5, run on a 16% tris-glycine gel. 1.10., M 12 Molecular Weight Standards; 2. 9., sulfitolyzed TnI; 3.0.05 mM DTT; 4. 0.10 mM DTT; 5. 0.2 mM DTT; 6. 0.3 mM DTT; 7. 0.5 mM DTT; 8. 1.0 mM DTT.

(¶ on page 13, lines 10-14)

During final ultrafiltration/diafiltration processing, product precipitation was noted. After removing final product, residual troponin precipitate in the UF/DF was resolubilized by washing with 15 mL of 6 M urea, 10 mM of sodium citrate, 0.15 m NaCl, ph 6. This resolubilzed troponin was buffer exchanged to remove urea and analyzed for troponin. The product total is the sum of the troponin recovered during the final UF/DF step and the resolubilzed, buffer exchange cassette wash.

(¶ on page 4, line 10)

Figure 10. Troponin I Lysate C mapping

(¶ on page 4, lines 16-26)

Troponin I from human cartilidge has recently been reported to possess antiangiogenic activity. In order to produce proteins to exploit the antiangiogenic properties of recombinant troponin I, we overexpressed the human skeletal troponin I Cdna in *E. coli*. Expression levels range from 2-10 mg/gram of wet cell paste. The

recombinant troponin I was isolated from the lysed cells in the inclusion bodies, which were solubilized and modified by sulfitolyzation of cysteine residues to improve protein processing. The sulfitolyzed protein was purified from inclusion bodies by sequential anion exchange and hydrophobic interaction chromatography. Cysteine protecting groups could be removed by reduction prior to final protein formulation. Overall yield of troponin from the multi-step purification was greater than 50% at purity levels greater than 95%. The purified recombinant human troponin I is structurally characterized, *e.g.*, by liquid chromatography/mass spectroscopy, peptide mapping, capillary electrophorisis, SEC with laser light scattering detection and SDS-PAGE.

(¶ on page 5, lines 14-20)

Recombinant TnI can be expressed in bacterial systems in a soluable form, or an insoluable form, in inclusion bodies. Recovery of TnI from inclusion bodies requires treatment with solubilzing protein denaturants like urea. In addition, TnI, is theoretical pI is 8.8, has limited solubility at ph values above 4 in the absence of chaotropic agents, although TnI is soluble at levels of 10-20 mg/ml at low ph (less than 3). High levels (1-6 M) of the protein denaturant urea are therefore ordinarily maintained during protein purification of TnI to ensure high solubility and good protein recovery.